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ORIGINAL ARTICLE

Evidence for a differential role of HPA-axis function, inflammation and metabolic syndrome in melancholic versus atypical depression

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The hypothalamic–pituitary–adrenal (HPA) axis and the inflammatory response system have been suggested as pathophysiological mechanisms implicated in the etiology of major depressive disorder (MDD). Although meta-analyses do confirm associations between depression and these biological systems, effect sizes vary greatly among individual studies. A potentially important factor explaining variability is heterogeneity of MDD. Aim of this study was to evaluate the association between depressive subtypes (based on latent class analysis) and biological measures. Data from 776 persons from the Netherlands Study of Depression and Anxiety, including 111 chronic depressed persons with melancholic depression, 122 with atypical depression and 543 controls were analyzed. Inflammatory markers (C-reactive protein, interleukin-6, tumor necrosis factor-α), metabolic syndrome components, body mass index (BMI), saliva cortisol awakening curves (area under the curve with respect to the ground (AUCg) and with respect to the increase (AUCi)), and diurnal cortisol slope were compared among groups. Persons with melancholic depression had a higher AUCg and higher diurnal slope compared with persons with atypical depression and with controls. Persons with atypical depression had significantly higher levels of inflammatory markers, BMI, waist circumference and triglycerides, and lower high-density lipid cholesterol than persons with melancholic depression and controls. This study confirms that chronic forms of the two major subtypes of depression are associated with different biological correlates with inflammatory and metabolic dysregulation in atypical depression and HPA-axis hyperactivity in melancholic depression. The data provide further evidence that chronic forms of depressive subtypes differ not only in their symptom presentation, but also in their biological correlates. These findings have important implications for future research on pathophysiological pathways of depression and treatment.

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Keywords: depression; inflammation; metabolic syndrome; salivary cortisol; subtypes

INTRODUCTION

Three often-studied pathophysiological systems that have a role in the etiology of major depressive disorder (MDD) are the hypothalamic–pituitary–adrenal (HPA) axis, the inflammatory response system and metabolic abnormalities. HPA-axis hyperactivity has been demonstrated in depressed persons compared with controls, and has been further implicated as a potential mechanism through which depression increases the risk of cardiovascular disease and other somatic diseases.[1] Alterations in the immune response system have been reported as well, with depressed persons having higher serum levels of pro-inflammatory cytokines such as C-reactive protein (CRP), interleukin (IL)-6 and tumor necrosis factor (TNF)-α compared with healthy individuals.[2–4] Related to this, more metabolic abnormalities such as obesity and adverse lipoprotein patterns are also associated with MDD,[5–7] and several studies have shown an association between depression and metabolic syndrome.[8,9]

Although meta-analyses have confirmed significant associations between depression and HPA-axis measures (cortisol, adrenocorticotropic hormone) and inflammation (CRP, IL-6, TNF-α), there is substantial variability in the effect sizes across studies.[1,2,10] Such variability could be attributable to sampling (for example, clinical sample versus community), composition of the sample (for example, age and ethnic composition) or to methodological differences in measures of depression and biological correlates. For example, previous studies have indicated that chronic depression, specifically dysthymia, may differ from non-chronic depression in levels of inflammatory markers.[11] However, variability could also be associated with the heterogeneity of the MDD diagnosis, particularly to differences in biological systems among those with different depressive subtypes. We hypothesize that the heterogeneity of MDD significantly contributes to this variability.

Some evidence in support of this hypothesis suggests that depressive subtypes contribute to variability in associations with biological measures. Subtypes represent more homogeneous groups of cases, and may potentially have different underlying pathophysiological processes. For instance, the association between melancholic depression and the HPA-axis hyperactivity has been replicated in some studies, whereas persons with

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atypical depression have been found to have a lower cortisol values than persons without atypical depression.[1,12,13] Atypical features were recently linked to decreased IL-4 and increased IL-2 compared with persons without atypical features in one study,[14] while another study reported decreased IL-2 in atypical depression compared with controls.[11] Another study found no differences in CRP, IL-6 and TNF-α between melancholic and atypical depression, and a higher CRP in atypical depression compared with controls in multivariable analyses.[15] Findings on inflammatory markers among those with melancholic depression have been contradictory; whereas one study reported higher IL-1β among melancholics,[16] others found lower IL-1β compared with non-melancholics.[17] Based on meta-analytic work, Howren *et al.*[3] concluded that body mass index (BMI) may interact with CRP and IL-6 to yield a potential tridirectional relationship between adiposity, inflammation and depression. The high BMI levels of those with atypical depression[18,19] may indicate a differential association between atypical depression with inflammation compared with melancholic depression, as was also postulated by Gold and Chrousos.[12]

In the past, substantial research has been devoted to the identification of subtypes of depression[20] based on clinical correlates, treatment response or observed symptom profiles. Opposed to following these paths, a more novel way of identifying subtypes has become available in the form of data-driven techniques, which result in more empirically based subtypes. Using such techniques, we previously observed subtypes closely, but not exactly, matching the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th Edition) atypical and melancholic depression.[18,21] In line with a previous finding that metabolic syndrome was associated with neurovegetative symptoms of depression,[22] we observed differences between atypical and melancholic depression, including higher rates of metabolic syndrome.[18] If more homogeneous subtypes have different biological profiles, this may help bring us closer to underlying etiologies, and can help direct future etiological research and treatment development. Although several researchers have addressed differences in biological measures across depressive subtypes, to our knowledge, no previous studies have rigorously evaluated different biological systems among depressive subtypes and controls simultaneously.

Our previous empirical work on the Netherlands Study of Depression and Anxiety (NESDA) sample distinguished two severe depressive subtypes that differed in depressive symptom profile—one resembling melancholic and one resembling atypical depression.[18,21] The aim of the current study was to compare different pathophysiological indicators of HPA-axis function, the inflammatory response system and metabolic syndrome across these two subtypes of MDD and healthy controls. We expect that melancholic depression will show more pronounced HPA-axis hyperactivity, whereas atypical depression will, in this respect, not differ from controls. Further, we expect that atypical depression will show a profile of immune activation and metabolic abnormalities compared with melancholic depression, whereas melancholic depression and controls will not differ from each other in this aspect.

MATERIALS AND METHODS

Sample

Data from NESDA were used.[23] NESDA is a longitudinal naturalistic cohort study, consisting of 2981 persons (18–65 yrs), including those with lifetime or current anxiety and/or depressive disorders ($n = 2329$; 78%) and healthy controls ($n = 652$; 22%).[23] Participants were recruited from the community ($n = 564$; 19%), primary care ($n = 1610$; 54%) and specialized mental health care ($n = 807$; 27%) from September 2004 through February 2007 at three study sites (Amsterdam, Groningen and Leiden). Approval of the study protocol was granted by the Ethical Review Boards of all the participating centers and all the participants gave written informed consent. Exclusion criteria were (1) a primary clinical diagnosis of psychotic disorder, obsessive compulsive disorder, bipolar disorder or severe addiction disorder and (2) not being fluent in Dutch. At baseline, participants were invited for a 4-h interview at one of the three study sites. Information was collected on psychopathology, demographic characteristics, physical and psychosocial functioning. It further included a blood draw, medical assessment, computer tasks, two self-administered questionnaires and salivary cortisol assessment. For the current study, we included 776 persons, including 233 persons with a current diagnosis of MDD whose subtype has been established (see below for details), and 543 controls without lifetime depressive or anxiety disorders.

Depressive subtypes

MDD was diagnosed with the Composite International Diagnostic Interview (CIDI) lifetime version 2.1,[24] which was conducted by trained clinical research staff. For the current study, we used two depressive subtypes, a Severe Melancholic ($n = 111$) and a Severe Atypical ($n = 122$) subtype, which were previously identified based on a latent transition analysis (LTA) of patients with persistent chronic depression. Chronic depression was defined as having a current (12-month) diagnosis of MDD at both the baseline and at the 2-year follow-up measurement.

A detailed description of the LTA analyses and subtypes can be found elsewhere.[21] In short, 10 depressive symptoms from the CIDI were used as indicator variables to identify depression subtypes at each measurement. In the first step of these data-driven analyses, persons with similar symptom-endorsement patterns were clustered in classes. In a second step, after identifying the best-fitting models for each time point, transitions over time were modeled in a longitudinal analysis. The analyses revealed three subtypes of depression that were labeled by the researchers based on their observed symptom patterns: a Severe Melancholic/Typical subtype (average prevalence across time points: 27.2%) characterized by decreased appetite and weight loss, a Severe Atypical subtype (32.2%) characterized by overeating and weight gain, and a Moderate subtype (40.5%) that was characterized by lower symptom probabilities and overall lower severity. Transition analyses showed that 76% of the sample endorsed the same subtype at both measurements, indicating that this group had a relatively stable depressive subtype. It should be noted that our labels for subtypes do not refer to the DSM-classifiers. However, inclusion of the symptoms of mood reactivity and interpersonal sensitivity in the current definition atypical depression has been debated,[25–28] and this was underlined in our latent class analysis (LCA) analysis showing that these two symptoms did not discriminate subtypes.[18] Also, other LCA studies have found similar symptom patterns with appetite and weight being the most discriminating symptoms, showing robustness of the identified subtypes.[19,29] Because our labels were used to describe the classes in previous work, we use the same labels here as well for consistency, but it is important to point out that our LCA-based subtypes of melancholic and atypical depression are, thus, not literally resembling DSM-classifications but are based on LCA-driven analyses. More information about the LTA method, identified subtypes and their correlates are provided in the Supplementary Materials.

In the current analyses, we included only persons with chronic depression and a stable depressive subtype over time for the following reasons. Firstly, stable subtypes are likely more homogeneous. Within chronic forms of depression some heterogeneity may still exist with different episodes having a different presentation or etiology,[30] but this can be avoided by including only persons with a stable clinical presentation. Secondly, by including persons with a chronic depression (that is, diagnoses present at two different measurements) we capture the more disabling, severe forms of depression. There is increasing awareness that single episodes may be distinct from recurrent and chronic forms, with the latter type being increasingly viewed upon as a chronic recurring condition characterized by high disability.[30,31] These chronic forms furthermore may have higher heritability than single episodes of depression.[32] Finally, having more homogeneous subtypes may bring us closer to the underlying etiologies of subtypes.

Severity may also be an important correlate for many biological markers that makes direct comparison of subtypes of different severity somewhat difficult. Therefore, we only included the Severe Melancholic and the Severe Atypical subtypes as they were of similar severity. Furthermore, melancholic and atypical depression are well recognized in the literature and are also currently included in DSM-IV. The controls used in the study were persons without lifetime depressive or anxiety disorders at both the baseline and 2-year assessment.

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Biological measures

Metabolic syndrome and BMI. Serum levels of triglycerides, high-density lipid (HDL) cholesterol and fasting plasma glucose were determined in plasma samples, for which blood samples were collected after an overnight fast. Blood samples were drawn into vacuum tubes between 0730–0930 hrs and kept frozen at $-80\,^{\circ}C$. Blood pressure, height, weight and waist circumference were measured during the medical assessment. Blood pressure was measured twice during supine rest on the right arm with the Omron M4-I, HEM 752A (Omron Healthcare Europe B.V., Hoofddorp, The Netherlands) and was averaged over the two measurements. In the analyses, positive outliers (mean $+/-$ 3 s.d.) were trimmed to the mean $+/-$ 3 s.d. (waist circumference $n=3$, triglycerides $n=5$, HDL $n=5$, systolic blood pressure $n=5$, diastolic blood pressure $n=2$, glucose $n=5$). Metabolic syndrome was defined according to the Adult Treatment Panel III.[33,34] The criteria are as follows: (1) waist circumference >102 cm in men or >88 cm in women, (2) triglycerides $\geqslant 1.7\,mmol\,l^{-1}$ ($150\,mg\,dl^{-1}$), (3) HDL cholesterol $<1.03\,mmol\,l^{-1}$ ($40\,mg\,dl^{-1}$) in men or $<1.30\,mmol\,l^{-1}$ ($50\,mg\,dl^{-1}$) in women, (4) blood pressure $\geqslant 130/85$ mm Hg or use of antihypertensives and (5) fasting glucose $\geqslant 6.1\,mmol\,l^{-1}$ ($110\,mg\,dl^{-1}$) or drug treatment for elevated glucose. Further, a count of the total number of positive criteria was constructed. BMI was calculated ($kg\,m^{-2}$).

Inflammatory markers. CRP and IL-6 were assayed at the Clinical Chemistry department of the VU University Medical Center. High-sensitivity plasma levels of CRP were measured in duplicate by an in-house ELISA based on purified protein and polyclonal anti-CRP antibodies (Dako, Glostrup, Denmark). The lower detection limit of CRP is $0.1\,mg\,l^{-1}$ and the sensitivity is $0.05\,mg\,l^{-1}$. Intra- and inter-assay coefficients of variation were 5% and 10%, respectively. Plasma IL-6 levels were measured in duplicate by a high-sensitivity enzyme-linked immunosorbent assay (PeliKine CompactTM ELISA, Sanquin, Amsterdam, the Netherlands). Comparison of IL-6 levels according to this assay with that of the IL-6 R&D array (R&D systems Minneapolis, MN, USA) in 77 random NESDA participants showed a correlation of 0.88, confirming comparability of these methods. The lower detection limit of IL-6 is $0.35\,pg\,ml^{-1}$ and the sensitivity is $0.10\,pg\,ml^{-1}$. Intra- and inter-assay coefficients of variation were 8% and 12%, respectively. Plasma TNF-α levels were assayed in duplicate at Good Biomarker Science, Leiden, the Netherlands, using a high-sensitivity solid phase ELISA (Quantikine HS Human TNF-α Immunoassay, R&D systems, Minneapolis, MN, USA). The lower detection limit of TNF-α is $0.10\,pg\,ml^{-1}$ and the sensitivity is $0.11\,pg\,ml^{-1}$. Intra- and inter-assay coefficients of variation were 10% and 15%, respectively.

Cortisol. At the baseline interview, participants were instructed to collect saliva samples at home on a regular day (preferably, a working day). This method has been shown to be a reliable and minimally intrusive method to assess the active, unbound form of cortisol.[35] Samples for the cortisol awakening response were obtained using Salivettes (Sarstedt AG and Co, Nümbrecht, Germany) at awakening (T1), and 30 (T2), 45 (T3) and 60 (T4) minutes later. Additionally, participants collected a sample at 1100 hrs.

Samples were stored in refrigerators and returned by mail. After receipt, Salivettes were centrifuged at 2000 *g* for 10 min, aliquoted and stored at $-80\,^{\circ}C$. Cortisol analysis was performed by competitive electro-chemiluminescence immunoassay (E170; Roche, Basel, Switzerland) as described by van Aken *et al*.[36] The functional detection limit was $0.07\,\mu g\,dl^{-1}$ and the intra-assay and inter-assay variability coefficients in the measuring range were $<10\%$. Values collected outside a margin of 5 min before or after the time protocol were recoded as missing. Persons using corticosteroids or who were pregnant or breastfeeding were excluded from analyses ($n=47$). Values that were larger than the mean + 2 s.d. were coded as missing ($n=24$).

For the cortisol awakening response, the area under the curve with respect to the increase (AUCi) and with respect to the ground (AUCg) were calculated using trapezoid formulas.[37] To calculate AUCi and AUCg, samples of at least three time points had to be available. For those with one missing sample ($n=26$), the missing value was imputed using a linear regression model, including information on the available three cortisol levels, age, sex, awakening time and smoking status.[38] To assess the diurnal cortisol slope, we calculated the slope mean decline per hour as: diurnal slope $=(T_{awakening}-T_{evening})/(\text{time } T_{evening}-\text{time } T_{awakening})$.[39]

Covariates and descriptive variables

Potential confounding variables that were considered included age, sex, educational level and smoking (yes/no). Models for cortisol AUCg and AUCi were additionally corrected for awakening time on the day of saliva collection.

To describe groups, we used several clinical characteristics, including severity of depressive symptoms as measured with the Inventory of Depressive Symptoms 30-item self-report,[40] age of onset assessed in the CIDI interview, duration of symptoms in the 4 years before baseline as measured with the Lifechart method[41] and family history of depression in first-degree relatives (not including offspring) based on self-report.[42] Antidepressant use was based on drug-container inspection of all the drugs used in the past month (on at least 50% of days), classified according to the World Health Organization Anatomical Therapeutic Chemical classification, and included selective serotonin reuptake inhibitors (SSRI; ATC code N06AB), serotonin-norepinephrine reuptake inhibitors (SNRI; N06AX16, N06AX21), tricyclic antidepressants (TCAs; N06AA) and tetracyclic antidepressants (TeCAs; N06AX03, N06AX05, N06AX11). Duration of antidepressant treatment was also assessed in number of months. Also, use of anti-inflammatory medication (M01A, M01B, A07EB, A07EC), statins (C10AA, C10B) and corticosteroids (H02, R03BA, R03AK, D07) were assessed. Diagnoses of cardiovascular disease and diabetes were based on self-report.

Statistical analyses

All analyses were performed in SPSS, version 19. In case of non-normal distributions, biological measures were log-transformed when necessary. First, means of metabolic syndrome variables, inflammatory markers and cortisol variables were compared across groups (controls, melancholic depression, atypical depression) using analyses of variance. In multivariable analyses we corrected for potential confounders. To test our hypotheses, we tested differences between atypical and melancholic depression. We also tested differences between atypical and melancholic depression versus controls, to evaluate whether they were different from controls (that is, inflammation and metabolic syndrome in atypical depression, and cortisol in melancholic depression) or similar to controls (that is, inflammation and metabolic syndrome in melancholic depression, and cortisol in atypical depression). In addition, we calculated effect sizes (Cohen's *d*). For cortisol, we also analyzed the four cortisol awakening measurements in a linear mixed model, adjusting for age, sex, educational level, smoking and awakening time. As mixed models can handle missing data through maximum likelihood estimation, we included all persons who had at least two cortisol measurements available.

RESULTS

In Table 1, we describe the socio-demographic and clinical characteristics of the two LCA-based depressive subtypes and controls. Groups did not differ in age, but the depressed groups were more often female, had lower educational levels and were more often smokers. In terms of clinical characteristics, persons with melancholic depression had a slightly higher severity and chronicity than persons with atypical depression, but otherwise the depressed subtypes were comparable. With the exception of TCAs and TeCAs, used by only nine and seven persons, respectively, duration of treatment was relatively short. Duration of TeCAs was longer in atypical depressed persons. There were no differences in prevalence of diabetes and cardiovascular disease, statin use and corticosteroid use across groups, but the depressed groups used anti-inflammatory medication more often.

Unadjusted means and s.ds. for metabolic syndrome variables and BMI, inflammatory markers and cortisol are presented in Table 2. Of the metabolic measures, waist circumference, BMI and the number of metabolic syndrome criteria were significantly more common in atypical depression compared with controls and melancholic depression, and the levels of triglycerides were elevated in the atypical group compared with controls. Systolic blood pressure was significantly elevated in controls compared with both the depressive groups. CRP, IL-6 and TNF-α levels were most elevated in atypical depression. Of the cortisol measures, AUCg was significantly higher in melancholic depression compared with controls and atypical depression, while diurnal slope was lower in atypical depression compared with controls and melancholic depression.

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Table 1. Group characteristics of depressive subtype groups and controls

	N	*Controls*	*Melancholic*[a]	*Atypical*[a]	P-*value*
		N = *543*	N = *111*	N = *122*	
Demographics and health indicators					
Female (%)	776	60.6	65.8	79.5	<0.0001
Age, mean (s.d.)	776	41.3 (14.6)	40.2 (12.1)	39.6 (12.1)	0.40
Educational level (years), mean (s.d.)	776	12.9 (3.2)	10.8 (3.1)	11.3 (3.3)	<0.0001
Smoking (% yes)	776	24.7	59.5	32.8	<0.0001
Clinical characteristics					
Severity of depression, mean (s.d.)	233	NA	39.1 (10.0)	35.2 (11.5)	0.01
Age of onset, median (IQR)	233	NA	22.0 (17.0–34.0)	22.0 (16.0–32.0)	0.32
Duration sx(% time), median (IQR)	232	NA	0.40 (0.19–0.66)	0.29 (0.15–0.58)	0.09
Positive family history depression (%)	231	NA	81.7	82.8	0.82
Medication use baseline, *N* (%)[a]					
SSRI	233	NA	39 (35.1)	39 (32.0)	0.61
SNRI	233	NA	10 (9.0)	10 (8.2)	0.83
TCA	233	NA	4 (3.6)	5 (4.1)	0.85
Tetracyclic antidepressants	233	NA	4 (3.6)	3 (2.5)	0.61
Duration use among users (months), median (IQR)					
SSRI	78	NA	6 (3–35)	5 (2–35)	0.66
SNRI	20	NA	5.5 (2.5–24)	4 (2–30)	1.00
TCA	9	NA	20 (3.25–97.5)	36 (3.5–168)	0.73
Tetracyclic antidepressants	7	NA	3 (1.25–4.75)	72 (-[b])	0.06
Physical health indicators					
Medication use, *N* (%)					
Anti-inflammatory medication	776	8 (1.5)	6 (5.4)	4 (3.3)	0.03
Statins	776	33 (6.1)	5 (4.5)	10 (8.2)	0.50
Corticosteroids	776	24 (4.4)	5 (4.5)	7 (5.7)	0.82
Chronic diseases					
DM	776	22 (4.1)	5 (4.5)	8 (6.6)	0.49
CVD	766	20 (3.7)	4 (3.6)	8 (6.6)	0.63

Abbreviations: CVD, cardiovascular disease; DM, diabetes mellitus; IQR, interquartile range; NA, not applicable; SNRI, serotonin-norepinephrine reuptake inhibitors; SSRI, selective serotonin reuptake inhibitors; sx symptoms; TCA, tricyclic antidepressants.
[a]Subtypes based on latent class and latent transition analyses.
[b]IQR not available due to low numbers.

Table 2. Means (s.d.) of biological measures across stable depressive subtypes and controls (*N* = 776)

	N	*Controls*	*Melancholic*[a]	*Atypical*[a]	P-*value*	
		N = *543*	N = *111*	N = *122*		
Metabolic syndrome and BMI						
Waist circumference (cm)	774	88.1 (13.5)	86.2 (13.1)	94.0 (15.7)	<0.0001	B, C
Triglycerides (mmol l^{-1}) [b]	764	1.08 (1.67)	1.11 (1.68)	1.22 (1.62)	0.055	B
HDL cholesterol (mmol l^{-1})	760	1.63 (0.43)	1.59 (0.42)	1.55 (0.42)	0.16	
Systolic BP (mm Hg)	774	137.5 (19.9)	133.4 (17.3)	132.7 (15.9)	0.012	A, B
Diastolic BP (mm Hg)	774	80.9 (11.5)	80.8 (10.3)	81.4 (9.5)	0.91	
Fasting plasma glucose (mmol l^{-1})	766	5.13 (0.78)	5.13 (0.79)	5.21 (0.94)	0.62	
No. of MetSyn components, mean (s.d.)	765	1.4 (1.3)	1.4 (1.2)	1.8 (1.4)	0.01	B, C
BMI, mean (s.d.)	776	25.1 (4.6)	24.2 (4.8)	28.7 (6.0)	<0.0001	B, C
Inflammatory markers						
CRP (mg l^{-1}), mean (s.d.) [b]	768	1.12 (3.23)	1.18 (3.57)	1.86 (3.48)	<0.0001	B, C
IL-6 (pg ml^{-1}), mean (s.d.) [b]	769	0.73 (2.58)	0.75 (2.64)	1.04 (2.42)	0.001	B, C
TNF-α, (pg ml^{-1}), mean (s.d.)[b]	762	0.84 (1.90)	0.78 (1.89)	1.03 (1.97)	0.002	B, C
Cortisol						
AUCg	504	18.47 (6.85)	21.82 (8.34)	17.16 (6.13)	<0.0001	A, C
AUCi	504	1.62 (6.61)	3.27 (8.74)	2.90 (5.61)	0.10	
Diurnal cortisol slope	507	0.75 (0.46)	0.86 (0.51)	0.59 (0.34)	0.001	B, C

Abbreviations: AUCg, area under the curve with respect to the ground; AUCi, area under the curve with respect to the increase; BMI, body mass index; BP, blood pressure, CRP, C-reactive protein; HDL, high-density lipid; IL-6, interleukin-6; MetSyn, metabolic syndrome; TNF-α, tumor necrosis factor-alpha. A = Controls different from Melancholic. B = Controls different from Atypical. C = Atypical different from Melancholic.
[a]Subtypes based on latent class and latent transition analyses.
[b]Test on log-transformed variable, means are back-transformed.

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Table 3. Adjusted means (s.e.) of biological measures across depressive subtypes and controls ($N = 776$)

	Control	*Melancholic*[a]	*Atypical*[a]	*Overall* P-*value*	*Pairwise comparison* P-*value*		
	Mean (s.e.)	*Mean (s.e.)*	*Mean (s.e.)*		*Melancholic* versus *control*	*Atypical* versus *control*	*Atypical* versus *melancholic*
Metabolic syndrome components							
Waist circumference (cm)	87.9 (0.5)	85.5 (1.2)	95.5 (1.1)	<0.0001	0.07	<0.0001	<0.0001
Triglycerides (mmol l^{-1}) [b]	1.08 (1.02)	1.06 (1.05)	1.26 (1.04)	0.005	0.70	0.002	0.007
HDL cholesterol (mmol l^{-1})	1.63 (0.02)	1.63 (0.04)	1.52 (0.04)	0.022	0.96	0.007	0.04
Systolic BP (mm Hg)	137.1 (0.7)	133.2 (1.6)	134.6 (1.5)	0.05	0.03	0.14	0.50
Diastolic BP (mm Hg)	80.8 (0.4)	80.7 (1.0)	82.0 (0.9)	0.42	0.93	0.21	0.29
Fasting plasma glucose (mmol l^{-1})	5.11 (0.03)	5.13 (0.07)	5.27 (0.07)	0.09	0.83	0.03	0.13
No. of MetSyn components	1.4 (0.0)	1.3 (0.1)	1.8 (0.1)	<0.0001	0.11	0.001	<0.0001
BMI	25.1 (0.2)	23.9 (0.5)	28.8 (0.4)	<0.0001	0.02	<0.0001	<0.0001
Inflammatory markers							
CRP (mg l^{-1})[b]	1.18 (1.05)	1.05 (1.12)	1.67 (1.11)	0.005	0.37	0.004	0.003
IL-6 (pg ml^{-1})[b]	0.75 (1.04)	0.69 (1.09)	1.00 (1.09)	0.003	0.35	0.003	0.002
TNF-α, (pg ml^{-1})[b]	0.84 (1.03)	0.77 (1.07)	1.02 (1.06)	0.003	0.23	0.004	0.001
Cortisol							
AUCg[c]	18.44 (0.36)	21.57 (0.92)	17.47 (0.79)	0.002	0.002	0.27	0.001
AUCi[c]	1.73 (0.35)	2.80 (0.90)	2.73 (0.77)	0.34	0.27	0.24	0.95
Diurnal cortisol slope	0.74 (0.02)	0.90 (0.06)	0.62 (0.05)	0.001	0.01	0.04	<0.0001

Abbreviations: AUCg, area under the curve with respect to the ground; AUCi, area under the curve with respect to the increase; BMI body mass index; BP, blood pressure; CRP, C-reactive protein; HDL, high-density lipid; IL-6, interleukin-6; MetSyn, metabolic syndrome; TNF-α, tumor necrosis factor-alpha. Adjusted for sex, age, educational level and smoking.
[a]Subtypes based on latent class and latent transition analyses.
[b]Test on log-transformed variable, means are back-transformed.
[c]Additionally corrected for awakening time.

After adjustment for age, sex, educational level and smoking, a similar picture emerged with atypical depression having more metabolic abnormalities—including now lower HDL cholesterol also—and higher inflammatory markers. Those with atypical depression were also found to have a higher fasting glucose compared with controls. Persons with melancholic depression had a higher AUCg, indicating higher morning saliva cortisol levels (Table 3). Effect sizes for metabolic components were small, with the exception of waist circumference and BMI (Table 4). For these variables, large effect sizes were observed in the range of 0.63–1.03 for comparisons of atypical versus controls and melancholic. Small-to-moderate effect sizes were observed for the comparison of inflammatory markers in atypical versus controls (range 0.29–0.30) and for the comparison of atypical versus melancholic (range 0.39–0.42). For AUCg, effects sizes for the comparison of melancholic versus control and versus atypical were moderate with effect sizes of 0.45 and 0.59, respectively. The effect size of diurnal slope for melancholic versus atypical was of a moderate size (0.61).

Antidepressant use can affect levels of biological measures.[43–45] Although antidepressant use was similar across the two depressed groups, the comparisons with controls could be affected and therefore we ran additional analyses adjusting for SSRI, SNRI, TCA and TeCA. Results showed that patterns remained largely similar, although effect sizes were somewhat smaller. We also evaluated the possible influence of differences in duration of treatment and long-term treatment by excluding persons using TCAs and TeCAs ($n = 16$). Exclusion of these persons did not alter the results (data not shown).

To evaluate the role of BMI, we ran multivariable models with adjustment for BMI and found that between-group differences were no longer significant for CRP (overall $P = 0.99$) and IL-6 (overall $P = 0.37$; not tabulated). However, persons with atypical depression still had significantly higher TNF-α levels than controls ($P = 0.02$) and persons with melancholic depression ($P = 0.01$) after additional correction for BMI (not tabulated).

In an adjusted, linear mixed model analysis using the awakening cortisol measurements separately, persons with melancholic depression had a distinct higher morning awakening curve than persons with atypical depression and controls (Figure 1).

DISCUSSION

This study demonstrated distinct biological correlates of chronic forms of atypical and melancholic LCA-based subtypes of MDD. Results from this study confirm our hypotheses that HPA-axis hyperactivity was a distinct feature of persons with melancholic depression, whereas atypical depression was characterized by greater inflammation and metabolic abnormalities. These findings provide strong evidence that depressive subtypes based on data-driven techniques do not merely differ in symptom presentation but also have distinct biological characteristics that distinguish them from the other subtypes and from the non-depressed groups. This supports the notion that melancholic and atypical depressive subtypes may have different etiological pathways and/or consequences.

Our finding of hyperactivity of the HPA axis, as indicated by higher cortisol levels, in melancholic depression confirms the results of a recent meta-analysis finding that HPA hyperactivity was linked with melancholic features of depression.[1,13] However, the effect size of $d = 0.59$ in our study for the comparison of AUCg values in the melancholic versus atypical groups was larger than those reported by Stetler and Miller[1] for comparison of atypical versus non-atypical and melancholic versus non-melancholic

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Table 4. Effect sizes (95% CI)

	Cohen's d *(95% CI)*		
	Melancholic versus *control*	*Atypical* versus *control*	*Atypical* versus *melancholic*
Metabolic syndrome components			
Waist circumference (cm)	−0.20 (−0.40 to 0.01)	0.63 (0.43 to 0.83)	0.82 (0.55 to 1.09)
Triglycerides (mmol l^{-1})[a]	−0.04 (−0.25 to 0.16)	0.31 (0.11 to 0.51)	0.35 (0.09 to 0.61)
HDL cholesterol (mmol l^{-1})	0.00 (−0.21 to 0.21)	−0.28 (−0.48 to −0.08)	−0.28 (−0.54 to −0.02)
Systolic BP (mm Hg)	−0.24 (−0.44 to −0.04)	−0.15 (−0.35 to 0.04)	0.09 (−0.17 to 0.34)
Diastolic BP (mm Hg)	−0.01 (−0.22 to 0.19)	0.13 (−0.07 to 0.33)	0.14 (−0.12 to 0.40)
Fasting plasma glucose (mmol l^{-1})	0.03 (−0.18 to 0.23)	0.23 (0.03 to 0.42)	0.20 (−0.06 to 0.45)
No. of MetSyn components	−0.17 (−0.38 to 0.03)	0.36 (0.16 to 0.55)	0.52 (0.26 to 0.78)
BMI	−0.25 (−0.46 to −0.05)	0.78 (0.58 to 0.98)	1.03 (0.75 to 1.30)
Inflammatory markers			
CRP (mg l^{-1})[a]	−0.10 (−0.30 to 0.11)	0.29 (0.09 to 0.49)	0.39 (0.12 to 0.65)
IL-6 (pg ml^{-1})[a]	−0.10 (−0.31 to 0.10)	0.30 (0.10 to 0.50)	0.40 (0.14 to 0.66)
TNFα (pg ml^{-1})[a]	−0.13 (−0.34 to 0.07)	0.30 (0.10 to 0.50)	0.42 (0.16 to 0.68)
Cortisol			
AUCg	0.45 (0.17 to 0.73)	−0.14 (−0.39 to 0.10)	−0.59 (−0.93 to −0.24)
AUCi	0.16 (−0.12 to 0.44)	0.15 (−0.10 to 0.39)	−0.01 (−0.35 to 0.33)
Diurnal cortisol slope	0.34 (0.07 to 0.61)	−0.26 (−0.50 to −0.02)	−0.61 (−0.94 to −0.26)

Abbreviations: AUCg, area under the curve with respect to the ground; AUCi, area under the curve with respect to the increase; BP, blood pressure; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; HDL, high-density lipid; IL-6 interleukin-6; MetSyn, metabolic syndrome; TNF-α, tumor necrotic factor-alpha. Melancholic and atypical subtypes based on latent class and latent transition analyses.
[a]Log-transformed variable.

depression ($d = -0.34$ and $d = 0.22$, respectively). The lower cortisol and the lower diurnal cortisol slope in atypical depression compared with melancholic depression confirms that atypical depression is a distinct phenomenon, as suggested before.[1] Although persons with atypical depression did not have different AUCg and AUCi than controls, they had lower cortisol levels at awakening and a smaller diurnal slope.

Atypical depression was characterized by both metabolic disturbances and inflammation. Metabolic disturbances observed in atypical depression included higher BMI, waist circumference, triglycerides and lower HDL. Elevated levels of CRP, IL-6 and TNF-α were observed in atypical depression compared with both melancholic depression cases and controls. This is contradicting a recent finding of Yoon *et al.*,[14] who found no differences in IL-6 and TNF-α but observed increased IL-2 and decreased IL-4 levels in persons with atypical depression versus non-atypical depression. A recent study by Karlovic *et al.*,[15] including 32 persons with melancholic depression and 23 persons with atypical depression, also did not find differences between groups for CRP, TNF-α and IL-6 but did find higher CRP in atypical depression compared with controls. Explanations for the differences in findings may be the use of a different definition (based on LCA) in our study, smaller samples, use of clinical samples and poorer comparability of subtypes in terms of severity. As expected, we did not observe differences in inflammatory markers between the melancholic depression and the control group, suggesting that inflammation is limited to atypical depression. Although elevations in cytokine levels is said to unequivocally occur in melancholic depression as well, this could be an effect of severity. Many studies on melancholic depression include inpatients, but these persons usually have more severe depression (that is, higher severity, more comorbidity, more disability). In NESDA, the sample comprised of persons from the community, as well as from primary and secondary care, thus giving a fair representative of depressed persons in the community, as opposed to inpatient samples who are more likely to consist of only severe cases. Contrary to our expectations, persons with melancholic depression had lower systolic blood pressure and lower BMI than controls. Possible explanations for lower blood pressure include higher use of antihypertensive drugs and more heart failure among depressed persons, chronic low blood pressure may be a causal factor for depression and, lastly, depression and low blood pressure may have a common underlying factor.[46] Effects of nicotine on appetite may explain the lower BMI in the melancholic group,[47] as this group has the highest percentage of smokers.

The association between depression and obesity has also been widely reported with some evidence for a reciprocal link between depression and obesity.[5] As suggested previously,[29,48] the current results imply that atypical depression underlies the association between depression with obesity. Higher waist circumference can be caused by abdominal fat storage in the form of white adipose tissue, whose main function is the storage of triglycerides.[49] Together with the unfavorable lipid profiles, these results suggest that the metabolic dysregulations observed in atypical depression mainly involve the lipid/fat metabolism. The co-occurrence of metabolic disturbances and inflammation in atypical depression is not surprising, given that low-grade chronic inflammation is linked to obesity and the metabolic syndrome.[50–52] Inflammatory markers, including cytokines, are involved in many physiological domains that are relevant in depression, including neurotransmitter metabolism, and are known to induce sickness behavior, including depressive symptoms and symptoms of fatigue.[50,51,53] Furthermore, inflammation is now increasingly thought to have an important role in the development of depression[54] in what is called the 'cytokine hypothesis' of depression. Because adipose tissue produces cytokines, including IL-6 and TNF- α,[52] the state of inflammation in atypical depression is likely caused by the higher BMI in this subgroup. Additional models confirmed this, although the association between atypical depression and TNF-α was independent of BMI. This finding is in line with a smaller study of adults with clinical depression that found that BMI was partially, but not completely, responsible for increased inflammation.[55] Notably, a small study of depressed persons (of whom subtype was not mentioned) and BMI-matched controls found morning elevations of IL-6 in cases but no differences in morning cortisol.[4]

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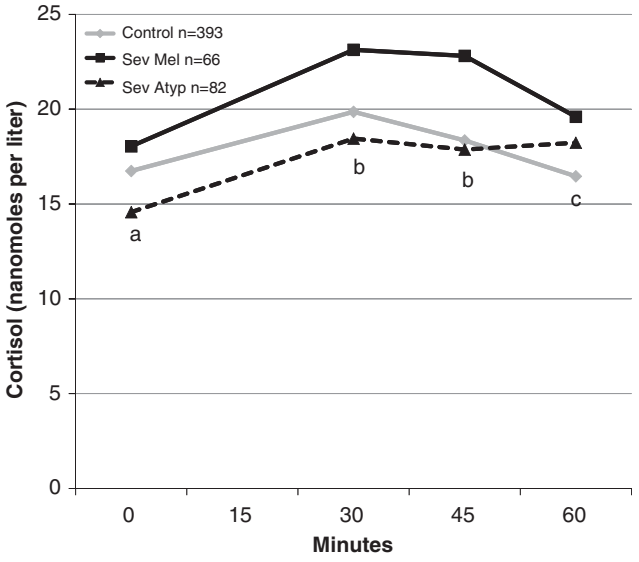

Figure 1. Cortisol morning awakening curve across depressive subtypes and controls. Mean salivary cortisol levels from a linear mixed model, adjusted for age, sex, educational level, smoking, body mass index and awakening time. Melancholic and atypical subtypes based on latent class and latent transition analyses: (**a**) mean cortisol of Severe Atypical (Sev Atyp) significantly lower than Severe Melancholic (Sev Mel) and controls ($P<0.05$); (**b**) mean cortisol of Severe Melancholic significantly higher than Severe Atypical and controls ($P<0.05$); and (**c**) mean cortisol of Severe Melancholic significantly higher than controls ($P<0.05$). Main effects of mixed model are as follows: For persons with Severe Melancholic versus Severe Atypical, $P=0.001$, interaction with time $P=0.45$. For persons with Severe Melancholic versus Controls $P=0.12$, interaction with time $P=0.13$. For persons with Severe Atypical versus Controls $P=0.008$, interaction with time $P=0.006$.

Although the found associations could indicate pathophysiological pathways, they could also represent epiphenomena. Although evidence for both pathophysiology and epiphenomena has been found for inflammation,[3] a larger body of evidence seems to indicate inflammation as a pathophysiological process.[3,51,56–60] For cortisol, evidence also points towards a pathophysiological process.[61] The current findings have important implications for future studies on pathophysiological mechanisms. Taking into account depressive subtypes in studies using newer biomarker techniques, such as cytokine microarrays and biomarkers pathway analyses, could bring us forward in the search for new biomarkers and treatments of depression. These results also suggest that stratification of analyses of new treatments by subtype may be informative in future studies.

In this study, we focused on HPA axis and inflammatory makers, but other biological systems may be helpful in distinguishing melancholic and atypical depressive subtypes. For instance, sleep-electroencephalogram studies have provided evidence for altered sleep in melancholic depression such as low slow-wave activity, short rapid eye movement latency and high rapid eye movement density, whereas in atypical depression, such alterations have not been reported.[62–64]

Several limitations should be considered when interpreting the results of this study. First, the cross-sectional character of this study does not allow for making causal inferences. Second, the study sample did not include inpatients. Third, it should be noted that the subtypes used were not based on DSM-IV criteria, but on more LCA/LTA classification methods. However, as especially the criteria for atypical depression have been criticized,[26,27,65] having subtypes based on more empirically based classification methods may be preferable. Fourth, we only included chronically depressed persons and therefore we can only generalize to chronic cases of depression.

To conclude, these findings provide evidence for heterogeneity of MDD based on pathophysiological measures; chronic forms of LCA-derived subtypes of melancholic and atypical depression have distinct biological correlates that may inform the etiology and treatment strategies for depression. Although hyperactivity of the HPA axis characterized melancholic depression, atypical depression is characterized by inflammation and metabolic abnormalities. These distinct pathophysiological indicators across depressive subtypes should aid future research on pathophysiological pathways and treatment of depression.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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